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APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
09/539,486	03/30/2000	SERGEY A. SELIFONOV	02-028940US	8406

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EXAMINER	
ZHOU, SHUBO	

ART UNIT	PAPER NUMBER
2653	

DATE MAILED: 12/04/2001

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/539,486	SELIFONOV ET AL.	
	Examiner	Art Unit	
	Shubo "Joe" Zhou	1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 September 2001*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-101* is/are pending in the application.

4a) Of the above claim(s) *47-92* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-29,31-38,40-46 and 93-101* is/are rejected.

7)☒ Claim(s) *30 and 39* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a)☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1)☒ Notice of References Cited (PTO-892)
2)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *19, 20, 14*
4)☐ Interview Summary (PTO-413) Paper No(s). _____.
5)☐ Notice of Informal Patent Application (PTO-152)
6)☐ Other

DETAILED ACTION

Applicant's amendment and request for reconsideration in Paper #18, filed on 9/20/01, is acknowledged and the amendments entered.

Applicant's arguments in response to the previous Office Action, mailed 3/15/01, have been fully considered but they are not deemed to be fully persuasive. Rejections and/or objections from previous Office actions not reiterated herein are hereby withdrawn. The following rejections and/or objections are either reiterated from the previous Office actions, or newly applied, and constitute the complete set presently being applied to the instant application.

Claims 1-46, and 93-101 are currently pending and under examination.

Claim Rejections-35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> **(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.**

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was

not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-4, 6-9, 12-29, 31-38, and 40-46, and the newly added claims 99-100, are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhao et al. (IDS document: Nature Biotechnology, Vol. 16:258-261, March, 1998) in view of Ho et al. (US patent No. 5023171, date of patent: Jun. 11, 1991).

Applicants amended claim 1 to recite "annealing the set of oligonucleotides to each other" and argue that Zhao et al. do not disclose that the oligos anneal to each other.

As set forth in the previous Office action (pages 4-10), Zhao et al. disclose a procedure for molecular evolution by staggered extension, an in vitro recombination process comprising providing a plurality of parental character strings corresponding to a plurality of nucleic acids, RC 1 and RC 2, which character strings, when aligned for maximum identity, comprises at least one region of heterology as listed in Table 1 (see page 258 and 259). Although Zhao et al. do not explicitly disclose the alignment of the sequences, it is obvious to artisans in the field that they must have done an alignment of the parental sequences in order to find the differences (heterology) listed in Table 1. A set of character string subsequences including differences as listed in Table 1 are defined (see page 258). A set of oligonucleotides corresponding to the set of character string subsequences are provided and annealed followed by elongation of one or more members of the set of oligonucleotides with a polymerase (see page 258 under "Results and discussion"). Since Zhao et al. use the procedure to create random recombinants between the two parental genes, the regions of the character strings include the regions of heterology and homology between the genes, as required in the instant claim 2. In

other words, the entire sequences of the genes are interpreted as the character strings as required in the instant claims. Since the two parental genes are two genes "encoding thermostable B. subtilis subtilisin E variants, each of which carries a single thermostable mutation along with several other neutral mutations" (see page 258, right column), it is obvious one of the two is an evolutionary intermediate, as required in the instant claim 3. Given the broad meaning of the word "design", the parental nucleic acids and the character strings are designed, as required in the instant claim 4. Figure 1 of Zhao et al. demonstrates the principle and procedure of the methodology which includes multiple cycles of denaturation, annealing and extension. Obviously, crossovers between the parental character strings are applied in the process, as required in the limitation of the instant claim 6.

Zhao et al. also disclose selection of multiple recombinants containing diplomat sequences, which diplomat sequences comprise intermediate level of sequence similarity between the two parental genes including the plurality of character strings (see page 261 and Figures 4 and 5 on page 260), as required in the instant claims 7 and 99. Zhao et al. select the mutations between the two variants as listed in Table 1 as desired crossover sites and disclose "priming the template sequences followed by repeated cycles of denaturation and extremely abbreviated annealing/polymerase-catalyzed extension" (see page 258, left column). Obviously, during the process, some oligonucleotides bridging these crossover sites are used in certain cycles of the procedure as evidenced by the sequences of the recombinant clones as shown in Figures 4 and 5 on page 260. Given the inherent broad meaning of the word "low", the similarity between the two parental character strings is low, as required in the instant claim 9, which strings contain the differences as listed in Table 1 in page 259.

Given the nature of Zhao et al.'s methodology, it is obvious that at different cycles of the procedure as shown in Figure 1, the "oligonucleotides" in the reactions are different in length and sequences, and they overlap each others, as required in the instant claim 12, e.g. the oligonucleotides shown in steps B and C of Figure 1, wherein the oligonucleotides in C overlap those in B. Zhao et al. disclose 10 positions in the parental genes including thermostable mutations and neutral mutations and use the positions to define segments along the genes and expect recombination between the positions. It is obvious that the character strings, i.e. the positions to be expected to be included in the resulting recombinants, will include different parts of the sequences between mutation sites, and therefore, the parental strings are segmented by these positions, as required in the instant claim 13. Although Zhao et al. do not disclose explicitly how the alignments between the parental genes/character strings are performed in order to find the differences as listed in Table 1, it is obvious to an ordinary artisan in the field that such alignments could be done with a digital computer or web-based system as being available to anyone in the field, as required in the instant claim 14.

As stated above, given the nature of the methodology disclosed by Zhao et al., it is obvious that at different cycles of the procedure as shown in Figure 1, different single stranded oligonucleotides are synthesized that correspond to the character strings subsequences including those mutations as disclosed in Table 1 and the single stranded oligonucleotides synthesized from one cycle are provided for the next cycle, as required in the instant claim 15. Also, it is obvious from Figure 1 and the "Experimental protocol" in page 261, that during the reactions at every cycle, the oligonucleotides synthesized in a previous step are pooled, hybridized and extended, wherein some of the extended double stranded nucleic acids comprise sequences from at least two of

the parental character strings including the mutations as listed in Table 1, as required in the instant claim 16. It is also obvious from Figure 1 and the "Experimental protocol" in page 261 that during the cycles including denaturation, annealing and extension, all the double stranded nucleic acids are denatured, a heterogeneous mixture of single-stranded nucleic acids are produced (as required in the instant claim 17), which heterogeneous mixture of single-stranded nucleic acids are re-hybridized and extended by the polymerase in the reaction mixture (as required in the instant claim 18). Such steps of denaturation, annealing and extension are repeated at least twice and can go up to around 80 (see page 261, left column), as required in the instant claim 19.

Zhao et al. disclose that the multiple recombinants resulted from the staggered extension process are selected since they confer higher activity (see Figure 3 in page 259), as required in the instant claim 20. Zhao et al. also disclose that six new point mutations were found in the 10 recombinants sequenced (see page 259, right column and Figure 5 in page 260), indicating that during the process, some oligonucleotides synthesized in the process comprise one or more replacement or alteration of the parental genes/character strings, as required in the instant claims 21 and 23. The procedure of selecting recombinants as disclosed by Zhao et al. includes extraction of DNA fragments of the size of the selected size (around 1 kb), which is interpreted as being that single stranded recombinants hybridize to other single stranded recombinants or parental nucleic acid that have a selected length to produce a pre-determined 1 kb of double stranded DNA (see page 261, left column), as required in the instant claim 24.

As stated above, given its broad meaning, it is interpreted that the two parental sequences, RC 1 and RC 2, as well as any strings that include the 10 mutations and/or the intervening sequences as disclosed by Zhao et al. are considered as parental

character strings. Also, all nucleic acids including those provided in cycle 1 and those synthesized in the reactions in the subsequent cycles that are less than the full-length parental genes are interpreted as oligonucleotides. As such, it is obvious from Figure 1 and the "Experimental protocol" in page 261 that the at least two parental character strings are provided, that the oligonucleotides provided and synthesized comprise at least one of oligonucleotide member comprising a chimeric nucleic acid sequence that comprises a subsequence from each of at least two parental character strings, wherein the subsequences from each parental character string are separated by a crossover point (as required in the instant claim 25), as evidenced by the sequences of the resulting recombinants listed in Figures 4 and 5 in page 260, wherein crossovers between the 10 mutations of the two parental genes are selected and such selection is random (as required in the instant claim 27) as shown in Figure 3 and the statement that "this distribution was similar to that expected for random recombination of the two phenotypic markers separated by 113 bp" (see page 259, right column). Yet, such crossovers are non-random (as required in the instant claims 28 and 29) in the sense that some very close markers/points are never recombined (see the second paragraph of the right column in page 259). It is also obvious that the crossover points including the 10 mutations as listed in Table 1 are selected by aligning the two parental character strings (as required in the instant claim 26) as stated above.

As mentioned above, all nucleic acids in the reactions disclosed by Zhao et al. including those provided in cycle 1 and those synthesized in the reactions in the subsequent cycles that are less than the full-length parental genes are interpreted as being oligonucleotides given the broad meaning of the word "oligonucleotide", it is obvious that at a particular cycle of the reaction, the concentration of some of the oligonucleotides are higher than that of others, which oligonucleotides with higher

concentrations are "added" to the next cycle, as required in the instant claim 31, and during the process of cycling, all the oligonucleotides are incubated with the recombinant nucleic acids and a polymerase (as required in the instant claim 32) in order for the cycling to continue. Therefore, in each cycle, the recombinant nucleic acid made from the last cycle is denatured and contacted with at least one additional nucleic acid from the oligonucleotides from the previous cycle (as required in the instant claim 33) and the cycle continues with annealing and extension.

Zhao et al. also disclose a brief description of a procedure referred to as DNA shuffling comprising, in addition to those as stated above, that "a set of parent genes is digested with DNase I to create a pool of short DNA fragments that are reassembled into full-length genes by repeated thermocycling in the presence of DNA polymerase" (see page 258, left column). It would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to combine the procedure of DNA shuffling and the staggered extension procedure as detailed above in that at certain cycle of the staggered extension procedure, at least one parental nucleic acid is cleaved by DNase I and the digested nucleic acid is contacted with the denatured recombinant nucleic acid (as required in the instant claims 34 and 35) synthesized in the previous cycle.

Both parental nucleic acids used in the procedures disclosed by Zhao et al. encode a bacterial gene product and the two initial oligonucleotides used in cycle 1 of the staggered extension procedure are 21 and 22 bp in length (see page 261), as required in the instant claims 36 and 37. As stated above, Zhao et al. select and provide several recombinant nucleic acids for their improved activity (as required in the instant claims 38) after sequencing and cloning (as required in the instant claims 42 and 43) and the improved activity is selected by an in vivo assay since the recombinant nucleic

acids are transferred into bacterial cells (as required in the instant claim 40). The plate assay can also be interpreted as an in vitro assay since the bacterial cells are positioned in an in vitro environment on a plate, as required in the instant claim 41 (see pages 258 and 261). Such recombinant nucleic acids are synthesized by staggered extension and the process is interpreted as being assembly PCR since it involves assembly and PCR, as required in the instant claim 44. In addition to staggered extension, Zhao et al. also disclose and compare other in vitro recombination methods including assembly PCR and error-prone assembly PCR and recombinant nucleic acids are selected and provided (see page 260, right column), as required in the instant claim 45. As stated repeatedly above, the parental strings disclosed by Zhao et al. include the 10 mutations, and it is obvious that these 10 mutations as listed in Table 1 are selected by aligning the two parental genes in a computer, as required in the instant claim 46.

There would have been a plethora of prior art references teaching the use of oligos that can be annealed to each other in the process of generating recombinant DNA. For example, Ho et al. teach of exactly such method of using oligonucleotide primers designed so that the ends of the resultant PCR products contain complementary sequences, and when the two PCR products are mixed, denatured and re-annealed, the single stranded DNA strands having the complementary sequences at their 3' ends anneal and then act as primers for each other. Ho et al. thus motivate application of primers that are annealable to each other in generating recombinant DNA.

Finally, claim 100 differs from claim 8 only in that the parental sequences in claim 100 are less than 50% similar. It would have been obvious that the methods of Zhao et al. can be used for sequences that are less than 50% similar, such as 49% similar, because the majority of the differences could lie outside where the primer sequences are. Further, Zhao et al. state that their method can be applied to directed evolution of

genes, operons, pathways, and even whole bacterial genomes (page 261), which obviously include sequences less than 50% similar.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to combine the teachings and/or motivations of Zhao et al. and Ho et al. to make and use the instant invention including using oligo primers that can be annealed to each other. There would have been an expectation of success because Zhao et al. and Ho et al. provided detailed experimental steps.

Claims 1-29, 31-38, 40-46 and 93-98, and the newly added claim 101, are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhao et al. (IDS document: Nature Biotechnology, Vol. 16:258-261, March, 1998) in view of Ho et al. (US patent No. 5023171, date of patent: Jun. 11, 1991) and Venkatasubramanian et al. (J. Chem. Inf. Comput. Sci. 1995, 33. 188-195), and in further view of Street et al. (IDS document: Structure, May 1999, 7:R105-R109) .

As shown above, Applicants amended claim 1 to recite "annealing the set of oligonucleotides to each other" and argue that Zhao et al. do not disclose that the oligos anneal to each other. Applicants also argue that no particular motivation is drawn from the references for combination thereof. This is not found persuasive. As set forth above, there would have been a plethora of prior art references teaching the use of oligos that can be annealed to each other in the process of generating recombinant DNA. For example, Ho et al. teach of exactly such method of using oligonucleotide primers designed so that the ends of the resultant PCR products contain complementary sequences, and when the two PCR products are mixed, denatured and re-annealed, the single stranded DNA strands having the complementary sequences at their 3' ends

anneal and then act as primers for each other. Ho et al. thus motivate application of primers that are annealable to each other in generating recombinant DNA.

Finally, claim 101 differs from claim 93 only in that the parental sequences in claim 101 are less than 50% similar. As set forth above, it would have been obvious that the methods of Zhao et al. can be used for sequences that are less than 50% similar, such as 49%, because the majority of the differences could lie outside where the primer sequences are. Further, Zhao et al. state that their method can be applied to directed evolution of genes, operons, pathways, and even whole bacterial genomes (page 261), which obviously include sequences less than 50% similar.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to combine the teachings and/or motivations of Zhao et al. and Ho et al. to make and use the instant invention including using oligo primers that can be annealed to each other. There would have been an expectation of success because Zhao et al. and Ho et al. provided detailed experimental steps. Further, as set forth in the previous Office action, Zhao et al. motivate and suggest computer simulation of recombination (page 11 of the previous Office action). This provides a particular motivation for combining the references of Zhao et al. with Venkatasubramanian et al. and Street et al., the latter two provide computer (in silico) methods for molecule design and recombination.

Claim Objections

Claims 30 and 39 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. This is reiterated from the previous Office action and maintained for reasons of record.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CFR § 1.6(d)). The CM1 Fax Center number is either (703) 308-4242 or (703)305-3014.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to:
Shubo "Joe" Zhou, Ph.D., whose telephone number is (703) 605-1158. The examiner can normally be reached on Monday-Friday from 8 A.M. to 4 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Woodward, Ph.D., can be reached on (703) 308-4028.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst Tina Plunkett whose telephone number is 703)-305-3524, or to the Technical Center receptionist whose telephone number is (703) 308-0196.

S. "Joe" Zhou, Ph.D.

Patent Examiner

MICHAEL BORIN, PH.D
PRIMARY EXAMINER